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Title of Project: Hemoglobin Metabolism and the Regulation of  
Erythrocyte Production and Destruction

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## Summary of Results

The biosynthesis of hemoglobin - In continuation of previous studies on the biosynthesis of heme, the conditions which influence the synthesis of heme in vitro in intact and lysed immature erythrocytes have been further defined. Studies on the effect of lead on heme synthesis indicate that the major, if not the sole, effect is an inhibition of the synthesis of proto-porphyrin. In an investigation of the possible role of folic acid in the biosynthesis of heme, it was found that aminopterin in large concentrations has no inhibitory effect and indeed may stimulate the synthesis of the porphyrin in vitro. Studies on the relative rates of synthesis of heme and of globin in immature erythrocytes derived from folic acid deficient turkeys are in progress. It has been established, however, that the synthesis of heme can progress relatively unimpaired in lysed immature erythrocytes whereas the synthesis of the globin portion is markedly diminished when the structural integrity of the erythrocyte is lost. This in vitro system provides a suitable technique for dissociating the synthetic processes of the pigment and protein portions of hemoglobin. Studies are similarly in progress on the effect of copper deficiency on the utilization of iron for heme synthesis. In the search for cofactors which may be involved in heme synthesis, we have found in yeast a heat-stable, non-coagulable, non-metallic substance which markedly diminishes the utilization of labeled glycine and of labeled acetate for heme synthesis. Work is in progress to determine whether this is an inhibitor or an intermediate in the biosynthesis of heme; the data are compatible with either interpretation.

In the course of some of this work, a method has been modified and developed for the isolation of glycine in pure form from relatively small amounts of protein. Conditions for the precipitation of glycine as the trioxalatochromiate have been defined; the subsequent treatment of this salt with ninhydrin and the formation of the dimedon derivative of formic acid provide a stable compound which is suitable for assaying the isotope activity

in the methylene carbon atom of glycine.

Origins of bile pigment. Previous studies have indicated that bile pigment is derived in part from one or more sources other than the hemoglobin of mature circulating erythrocytes. In attempting to define the nature of these sources, naturally occurring porphyrin compounds, mesoporphyrin IX, deuteroporphyrin IX, and hematoporphyrin IX labeled with  $C^{14}$  are being prepared for the study of their relationship to the synthesis of bile pigment.

Ageing of erythrocytes. In investigating the mechanism of ageing in human erythrocytes, we have been studying the metabolic behavior of various constituents of the erythrocyte. Previous studies have shown that the hemoglobin of the mature erythrocyte is metabolically in a stable state. The cholesterol of the human erythrocyte is in a dynamic state and is in equilibrium with the free cholesterol of the plasma (see appended reprint). Of particular pertinence to the problem of ageing of erythrocytes is the metabolic behavior of the glutathione of the red cell. Various data point to an important role for glutathione in maintaining the integrity of the erythrocyte. We have found that human, rabbit, and avian erythrocyte in vitro incorporate isotopic glycine into their glutathione at a rapid rate. Furthermore, this incorporation occurs in lysed as well as in intact cells and occurs in the post-hemolytic residue of the erythrocyte and not in the soluble supernatant portion of the hemolysate. In a study conducted in a normal man with the use of  $N^{15}$  labeled glycine, the glutathione of the mature human erythrocyte is found to be in the dynamic state and the nitrogen of the glutathione derived from the labeled glycine has a rapid turnover with a half-life time of approximately four days. Studies are now in progress to determine whether the incorporation of glycine into

glutathione represents net synthesis in the erythrocyte. The relationship of the metabolism of glutathione to glycolysis and to the ageing of the erythrocyte is under study.

REPORTS AND PUBLICATIONS:

1. Erythrocyte Metabolism. The Metabolic Behavior of the Cholesterol of Human Erythrocytes. I. M. London and H. Schwarz. J. Clin. Invest. 32, 1248, 1953
2. Erythrocyte Metabolism. Studies on the Metabolic Behavior of Reduced Glutathione in Human and Avian Erythrocytes. E. Dimant, E. Landsberg and I. M. London, New York, N. Y.

Abstract to appear in Proceedings of American Society for  
Clinical Investigation, J. Clin. Invest. June 1954.

Erythrocyte Metabolism. Studies on the Metabolic Behavior of  
Reduced Glutathione in Human and Avian Erythrocytes. Elhanan  
Dimant, Edith Landsberg and Irving M. London, New York, N. Y.

In studies on the ageing process in erythrocytes, the patterns of  
metabolic behavior of various constituents of the erythrocytes are under in-  
vestigation. This report is concerned with reduced glutathione, which has  
been implicated in the maintenance of integrity of the structure of the  
erythrocyte.

Glycine labeled with  $N^{15}$  was administered to a normal man, and gluta-  
thione and hemin were isolated from the erythrocytes at intervals thereafter.  
The isotope concentrations in the hemin reveal a normal pattern of survival  
of erythrocytes. The isotope concentrations in the reduced glutathione indicate  
that the nitrogen, derived from glycine, of the reduced glutathione in  
the human erythrocyte is in the dynamic state with a half-time value of  
approximately four days.

Incubation, in vitro, of intact or lysed human or duck erythrocytes with  
 $2-C^{14}$ -glycine results in rapid incorporation of the labeled carbon in the  
reduced glutathione of the erythrocytes. These findings indicate the existence  
of a system in the mature erythrocyte for continued replacement of the glycine  
moiety of reduced glutathione and provide a basis for the study of the origins,  
and the mechanism of reduction, of glutathione of erythrocytes.